(54) **Detection of HPV-induced invasive cancers and their precursor lesions with invasive potential**

(57) The invention is in the field of medicine and is concerned with a molecular diagnostic marker for HPV-induced invasive cancers and their precursor lesions with invasive potential. In particular the present invention relates to the use of the TSLC1 gene as marker for invasive cervical cancer. The invention provides methods for detecting HPV-induced invasive cancers and their precursor lesions associated with tumor suppressor lung cancer 1 (TSLC1), comprising contacting a target cellular component of a test cell with a reagent that detects TSLC1 and detecting a reduction in the TSLC1 as compared to that of a comparable normal cell. The invention also provides molecular diagnostic markers for premalignant cervical lesions with invasive potential associated with tumor suppressor lung cancer 1 (TSLC1) in cytologically abnormal cervical smears and/ or biopsies.

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Description

FIELD OF THE INVENTION

[0001]   The invention is in the field of medicine and is concerned with a molecular diagnostic marker for human papillomavirus (HPV)-induced invasive cancers and their precursor lesions such as invasive cervical cancer, premalignant cervical lesions with invasive potential and high-risk human papillomavirus (HPV)-induced non-cervical invasive cancers. In particular the present invention relates to the use of the *TSLC1* gene as marker for HPV-induced invasive cancers and their precursor lesions.

BACKGROUND OF THE INVENTION

[0002]   Cancer of the uterine cervix is the second most common cancer in women world-wide and is responsible for approximately 250.000 cancer deaths a year.

[0003]   Cervical cancer development is characterized by a sequence of premalignant lesions, so called cervical intraepithelial neoplasia (CIN), which are graded I to III, referring to mild dysplasia (CIN I), moderate dysplasia (CIN II) and severe dysplasia/carcinoma *in situ* (CIN III).

[0004]   Over the past decade it has been well established that cervical carcinogenesis is initiated by an infection with high-risk human papillomavirus (HPV). Expression of the viral oncogenes E6 and E7, which disturb the p53 and Rb tumor suppressor pathways, respectively, has been shown to be essential for both the onset of oncogenesis and the maintenance of a malignant phenotype. However, consistent with a multistep process of carcinogenesis, additional alterations in the host cell genome are required for progression of an hr-HPV infected cell to an invasive carcinoma.

[0005]   In line with multiple events underlying cervical carcinogenesis is the observation that only a small proportion of women infected with high-risk HPV will develop high-grade premalignant cervical lesions (CIN III) or cervical cancer, and in most women with premalignant cervical lesions the lesions regress spontaneously.

[0006]   However, at present no markers exist to predict which premalignant lesions will regress or ultimately progress to cervical cancer. Therefore, general medical practice comprises the treatment of all women with morphologically confirmed CIN II and CIN III, in order to prevent the development of cervical cancer. Consequently, many women are unnecessarily treated and unnecessarily worried.

SUMMARY OF THE INVENTION

[0007]   It has now surprisingly been found that the tumor suppressor lung cancer 1 gene (*TSLC1*) is involved as a tumor suppressor gene in cervical carcinogenesis and that *TSLC1* silencing, or a low level of expression of the *TSLC1* gene, is an important determinant of cervical carcinogenesis. Compared to its role in the development of a subset of lung carcinomas, the silencing of *TSLC1* plays an even more predominant role in cervical carcinogenesis. *TSLC1* and the gene products thereof thus provide valuable molecular markers to diagnose invasive cervical lesions and/or to (better) predict which premalignant lesions have a high chance of progressing to cervical cancer.

[0008]   Cervical cancer is almost exclusively associated with human papillomavirus (HPV) infection. Human papillomaviruses, constitute a group of more than 100 types of viruses, as identified by variations in DNA sequence. The various HPVs cause a variety of cutaneous and mucosal diseases. Certain types may cause warts, or papillomas, which are benign (noncancerous) tumors. Others have been found to cause invasive carcinoma of the uterine cervix.

[0009]   HPVs are broadly classified into low-risk and high-risk types, based on their ability to induce malignant changes in infected cells. Low risk HPV types such as 1, 2, 4, 6, 11, 13 and 32 are primarily associated with benign lesions or common warts while the high risk types, such as 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 are primarily associated with premalignant and malignant epithelial lesions. These high-risk types of HPV cause growths that are usually flat and nearly invisible, as compared with the warts caused by low-risk types, e.g. HPV-6 and HPV-11.

[0010]   Therefore, the present invention is not only suited to detect invasive cervical cancer associated with tumor suppressor lung cancer 1 (TSLC1), but also other invasive cancers that are induced by HPV, particularly of the high-risk type and provides a method for the risk assessment of premalignant lesions becoming invasive.

[0011]   Accordingly, the present invention provides methods of detecting HPV-induced invasive cancers and their precursor lesions associated with tumor suppressor lung cancer 1 (TSLC1) in a subject in need thereof, said method comprising contacting a cell component of a test cell of the subject with a reagent that detects the level of the cell component in the test cell and determining a modification in the level of the cell component in the test cell as compared with a comparable healthy cell, wherein the cell component indicates the level of TSLC1 in the cell and the modification indicates the presence of HPV-induced invasive cancer.

[0012]   Very suitable HPV-induced invasive cancers or precursor lesions thereof in the context of the present invention are invasive cervical cancers and premalignant cervical lesions with invasive potential, but also invasive cancers and

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premalignant lesions that are induced by HPV in other tissues such as from oral cavity, oropharynx, anus, rectum, penis, vulva, etc.

[0013] A test cell may be a neoplastic cell, a proliferating cervical cell, or any other cell wherein the presence of an HPV-induced invasive cancer or precursor lesion thereof associated with tumor suppressor lung cancer 1 is to be detected.

[0014] In another embodiment, the present invention provides methods of detecting HPV-induced invasive cancers and their precursor lesions associated with tumor suppressor lung cancer 1 (TSLC1) in a subject in need thereof, said method comprising contacting a target cellular component of a test cell with a reagent that detects TSLC1 and detecting a reduction in the TSLC1 as compared to that of a comparable normal cell, preferably in said detection an increased methylation of the *TSLC1* promoter in the test cell and/or a reduced production of TSLC1 in the test cell as compared to the comparable normal cell is determined.

[0015] In yet another embodiment, the present invention provides methods of treating high-risk HPV-induced invasive cancers and their precursor lesions associated with modification of TSLC1 production in test cells in a subject in need thereof, said method comprising contacting cells of a patient suffering from said cancer with a therapeutically effective amount of a reagent that increases TSLC1 level in test cells.

[0016] In another aspect, the present invention relates to the use of molecular diagnostic markers for the detection of HPV-induced invasive cancers and their precursor lesions associated with tumor suppressor lung cancer 1 (TSLC1), wherein said marker indicates *TSLC1* promoter methylation, expression of mRNA associated with production of TSLC1 polypeptide and/or allelic loss of the 11q23 chromosome. By such use, the risk of progression to invasive cancer may be determined.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figure 1 shows the *TSLC1* 5' regulatory region (-894 to -1) and coding and transcribed 3' non-coding sequences (1 to 1456), derived from Genbank accession codes AP_003172 and NM_014333, respectively. The start and stop codons are represented in italics and are underlined

DETAILED DESCRIPTION OF THE INVENTION

[0018] "Expression" refers to the transcription of a gene into structural RNA (rRNA, tRNA) or messenger RNA (mRNA) and, if applicable, subsequent translation into a protein.

[0019] The term "HPV-induced invasive cancer" refers to a carcinoma induced by high-risk HPV, which invades surrounding tissue.

[0020] The term "Invasive cervical cancer" refers to a cervical carcinoma invading surrounding tissue.

[0021] The terms "premalignant lesion" and "precursor lesion" refer to a stage in the multistep cellular evolution to cancer with a strongly increased chance to progress to a carcinoma. With classical morphology the pathologist is unable to predict in the individual patient which of these lesions will progress or regress. The current invention refers to a method, which can predict the progression to invasive cancer.

[0022] The term "invasive potential" refers to the potential to invade surrounding tissue and consequently to become malignant.

[0023] The term "premalignant cervical lesion" refers to a stage in the multistep cellular evolution to cervical cancer with a strongly increased chance to progress to a cervical carcinoma. With classical morphology the pathologist is unable to predict in the individual patient which of these lesions will progress or regress

[0024] The term "capable of specifically hybridizing to" refers to a nucleic acid sequence capable of specific base-pairing with a complementary nucleic acid sequence and binding thereto to form a nucleic acid duplex.

[0025] A "complement" or "complementary sequence" is a sequence of nucleotides which forms a hydrogen-bonded duplex with another sequence of nucleotides according to Watson-Crick base-paring rules. For example, the complementary base sequence for 5'-AAGGCT-3' is 3'-TTCCGA-5'.

[0026] The term "stringent hybridization conditions" refers to hybridization conditions that affect the stability of hybrids, e.g., temperature, salt concentration, pH, formamide concentration and the like. These conditions are empirically optimised to maximize specific binding and minimize non-specific binding of the primer or the probe to its target nucleic acid sequence. The terms as used include reference to conditions under which a probe or primer will hybridise to its target sequence, to a detectably greater degree than other sequences (e.g. at least 2-fold over background). Stringent conditions are sequence dependent and will be different in different circumstances. Longer sequences hybridise specifically at higher temperatures. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridises to a perfectly matched probe or primer. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M Na

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ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes or primers (e.g. 10 to 50 nucleotides) and at least about 60°C for long probes or primers (e.g. greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringent conditions or "conditions of reduced stringency" include hybridization with

5   a buffer solution of 30% formamide, 1 M NaCl, 1% SDS at 37°C and a wash in 2x SSC at 40°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1x SSC at 60°C. Hybridization procedures are well known in the art and are described in e.g. Ausubel et al, Current Protocols in Molecular Biology, John Wiley & Sons Inc., 1994.

[0027] The term "oligonucleotide" refers to a short sequence of nucleotide monomers (usually 6 to 100 nucleotides)

10 joined by phosphorous linkages (e.g., phosphodiester, alkyl and aryl-phosphate, phosphorothioate), or non-phosphorous linkages (e.g., peptide, sulfamate and others). An oligonucleotide may contain modified nucleotides having modified bases (e.g., 5-methyl cytosine) and modified sugar groups (e.g., 2'-O-methyl ribosyl, 2'-O-methoxyethyl ribosyl, 2'-fluoro ribosyl, 2'-amino ribosyl, and the like). Oligonucleotides may be naturally-occurring or synthetic molecules of double-and single-stranded DNA and double- and single-stranded RNA with circular, branched or linear shapes and

15 optionally including domains capable of forming stable secondary structures (e.g., stem-and-loop and loop-stem-loop structures).

[0028] The term "primer" as used herein refers to an oligonucleotide which is capable of annealing to the amplification target allowing a DNA polymerase to attach thereby serving as a point of initiation of DNA synthesis when placed under conditions in which synthesis of primer extension product which is complementary to a nucleic acid strand is induced,

20 i.e., in the presence of nucleotides and an agent for polymerization such as DNA polymerase and at a suitable temperature and pH. The (amplification) primer is preferably single stranded for maximum efficiency in amplification. Preferably, the primer is an oligodeoxy ribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the agent for polymerization. The exact lengths of the primers will depend on many factors, including temperature and source of primer. A "pair of bi-directional primers" as used herein refers to one

25 forward and one reverse primer as commonly used in the art of DNA amplification such as in PCR amplification.

[0029] The term "probe" refers to a single-stranded oligonucleotide sequence that will recognize and form a hydrogen-bonded duplex with a complementary sequence in a target nucleic acid sequence analyte or its cDNA derivative.

[0030] The TSLC1 (tumor suppressor in lung cancer 1) gene (Genbank Accession NM_014333) has originally been identified as a tumor suppressor gene in the lung cancer cell line A549 by functional complementation studies (Kuramo-

30 chi et al., 2001; Nature Genet 27, 427-430). It was shown that re-expression of TSLC1 in the lung cancer cell line A549 suppressed tumorigenicity in nude mice. Moreover, loss or suppression of TSLC1 expression in other lung cancer cell lines was shown to be correlated to both tumorigenicity and spleen to liver metastasis in nude mice. TSLC1 mRNA suppression in these cell lines was found to correlate to hypermethylation of the TSLC1 promoter.

[0031] Subsequent analysis of non-small cell lung cancers (NSCLC) revealed allelic loss at 11q23.2, the TSLC1

35 locus, in about 40% of lung cancers and within this group of tumors with allelic loss promoter hypermethylation of the other allele could be detected in 85% of cases.

[0032] TSLC1 encodes a member of the immunoglobulin super family of cell adhesion molecules (IgCAMs), which consists of a wide variety of cell-surface molecules that are characterized by an immunoglobulin unit. The TSLC1 protein is an N-linked glycoprotein of 75 kDa, which is localized at the cell membrane and is involved in intracellular

40 adhesion through homophilic trans-interaction (Masuda et al, 2002; J Biol Chem 277, 31014-31019).

[0033] The present inventors have now established that TSLC1 silencing is a frequent event in cervical cancer cell lines. TSLC1 silencing is found to result from TSLC1 promoter hypermethylation either or in not in combination with allelic loss. In vitro studies revealed a functional involvement of TSLC1 inactivation in both anchorage independent growth and tumorigenicity of cervical cancer cells, whereas immortality and proliferation were not affected. This points

45 to a role for TSLC1 silencing in tumor invasion rather than proliferation. Loss of a putative tumor suppressor gene on chromosome 11 was already known to be involved in tumorigenicity of SiHa cervical cancer cells, and allelic loss at 11q22-23 has frequently been detected in invasive cervical carcinoma. These results indicate that inactivation of TSLC1 at 11q23.2 might play a crucial role in cervical cancer invasion.

[0034] Analysis of cervical tissue specimens revealed that TSLC1 promoter hypermethylation is limited to only a

50 subset of high-grade CIN lesions but detectable in up to 58% of invasive cervical carcinomas. Moreover, TSLC1 promoter hypermethylation can specifically be detected in cervical smears derived from women with cervical cancer. Analysis of cervical cancer cell lines revealed that silencing of TSLC1 expression is present in as much as 91% (10/11) of cell lines. TSLC1 silencing appeared not only associated with promoter methylation, but also with allelic loss at the TSLC1 locus and yet unknown events, suggesting that the percentage of cervical cancers showing TSLC1 promoter

55 methylation is even an underestimation of the actual percentage of cases in which the TSLC1 gene is silenced.

[0035] Accordingly, the present invention provides methods of detecting HPV-induced invasive cancers and their precursor lesions associated with tumor suppressor lung cancer 1 (TSLC1) in a subject in need thereof, or indicative thereof, said method comprising contacting a cell component of a test cell of the subject with a reagent that detects

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the level of the cell component in the test cell and determining a modification in the level of the cell component in the test cell as compared with a comparable healthy cell, wherein the cell component indicates the level of TSLC1 in the cell and the modification indicates the presence of HPV-induced invasive cancers and their precursor lesions.

[0036] The test cell of the subject may comprise a cell from a sample of skin cells (e.g. in the case of warts, veruccas and the like; presumably caused by cutaneous HPV infections), a sample of mucosal cells, such as cervical cells, and also other tissue such as oral cavity, oropharynx, , penis, vulva, anus, rectum and other tissues wherein a cancer associated with HPV is to be detected. All such samples may be used as a sample in a method of the present invention. Preferably, a sample of a patient's cells comprise cervical cells as test cells.

[0037] A method of the present invention is particularly suited for the detection of HPV-induced invasive cancers and their precursor lesions associated with tumor suppressor lung cancer 1 (TSLC1) that are induced by high-risk HPVs. A method of detecting HPV-induced invasive cancers and their precursor lesions associated with tumor suppressor lung cancer 1 (TSLC1) may accordingly relate to the measurement of TSLC1 expression, such as in the form of measuring TSLC1 gene transcripts and/or subsequent proteins translated from said transcripts. Also a method of detecting HPV-induced invasive cancers and their precursor lesions may comprise measuring TSLC1 promoter methylation as an indication of TSLC1 expression capacity and/or TSLC1 protein production capacity.

[0038] Figure 1 shows the cg-rich promoter region upstream of the atg start codon in the TSLC1 gene and the coding region for the TSLC1 protein. Methylation of the cg-rich promoter region will result in a sharply decreased transcription or even complete blockage of transcription. Therefore, the promoter region provides a positive marker sequence for the expression potential of this gene. Alternatively, the expression of the TSLC1 gene may be detected by measuring gene transcripts. As such, the coding region for the TSLC1 protein in this gene provides a marker sequence for detection of transcripts of the gene. In yet another alternative, the expression of the TSLC1 gene may be detected by measuring TSLC1 protein directly.

[0039] The test cell component contacted can thus be nucleic acid, such as DNA or RNA, preferably mRNA, or protein. When a cell component is protein, the reagent is typically an anti-TSLC1 antibody. When the component is nucleic acid, the reagent is typically a nucleic acid (DNA or RNA) probe or (PCR) primer. By using such probes or primers, gene expression products, such as mRNA may for example be detected. Alternatively, when the component is nucleic acid, the reagent may also be a restriction endonuclease, preferably a methylation sensitive restriction endonuclease for the detection of the presence of methyl groups on the test cell nucleic acid, said test cell nucleic acid then preferably being DNA.

[0040] The test cell component may be detected directly in situ or it may be isolated from other cell components by common methods known to those of skill in the art before contacting with the reagent (see for example, "Current Protocols in Molecular Biology", Ausubel et al. 1995. 4th edition, John Wiley and Sons; "A Laboratoty Guide to RNA: Isolation, analysis, and synthesis", Krieg (ed.), 1996, Wiley-Liss; "Molecular Cloning: A laboratory manual", J. Sambrook, E.F. Fritsch. 1989. 3 Vols, 2nd edition, Cold Spring Harbor Laboratory Press)

[0041] Detection methods include such analyses as Southern and Northern blot analyses, RNase protection, immunoassays, in situ hybridization, PCR (Mullis 1987, U.S. Pat. No. 4,683,195, 4,683,202, en 4,800,159), LCR (Barany 1991, Proc. Natl. Acad. Sci. USA 88:189-193; EP Application No. 320,308), 3SR (Guatelli et al., 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878), SDA (U.S. Pat. Nos. 5,270,184, en 5,455,166), TAS (Kwoh et al., Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi et al., 1988, Bio/Technology 6:1197), Rolling Circle Amplication (RCA) or other methods for the amplification of DNA. In an alternative method RNA may be detected by such methods as NASBA (L. Malek et al., 1994, Meth. Molec. Biol. 28, Ch. 36, Isaac PG, ed., Humana Press, Inc., Totowa, N.J.) or TMA.

[0042] Nucleic acid probes, primers and antibodies can be detectably labeled, for instance, with a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme. Those of ordinary skill in the art will know of other suitable labels for binding to the reagents or will be able to ascertain such, using routine experimentation.

[0043] Other methods for detection include such analyses as can be performed with nucleic acid arrays (See i.a. Chee et al., 1996, Science 274(5287):610-614). For example, DNA arrays may be used for the detection of nucleic acids according to the invention. Such arrays comprise oligonucleotides with sequences capable of hybridizing under stringent conditions to the nucleic acid cell component of which the level is detected in a method of the present invention.

[0044] Since the present invention shows that a decreased level of TSLC1 transcription is often the result of hypermethylation of the TSLC1 gene, it is often desirable to directly determine whether the TSLC1 gene is hypermethylated. In particular, the cytosine rich areas termed "CpG islands", which lie in the 5' regulatory regions of genes are normally unmethylated. The term "hypermethylation" includes any methylation of cytosine at a position that is normally unmethylated in the TSLC1 gene sequence (e. g. the TSLC1 promoter). Hypermethylation can for instance be detected by restriction endonuclease treatment of the TSLC1 polynucleotide (gene) and Southern blot analysis. Therefore, in an invention method wherein the cellular component detected is DNA, restriction endonuclease analysis is prefered to detect hypermethylation of the TSLC1 gene. Any restriction endonuclease that includes CG as part of its recognition

site and that is inhibited when the C is methylated, can be utilized. Methylation sensitive restriction endonucleases such as BssHII, MspI, NotI or HpaII, used alone or in combination, are examples of such endonucleases. Other methylation sensitive restriction endonucleases will be known to those of skill in the art.

[0045]   Other methods for the detection of *TSLC1* promoter hypermethylation involve bisulfite modification of DNA, in which the unmethylated cytosines are converted to an uracil whereas the methylated cytosines are protected from chemical modification. Subsequent PCR amplification and sequencing will reveal whether cytosines in CpG islands are maintained in case of methylation or replaced by a uracil in case of an unmethylated status. Another method involves the treatment a PCR amplified product generated from bisulfite modified DNA with restriction endonuclease that includes CG as part of its recognition site.

[0046]   An alternative means to test for methylated sequences is a methylation specific PCR, which is also based on bisulfite modification of DNA, followed by specific PCR reactions that target CpG rich sequences.

[0047]   For purposes of the invention, an antibody (*i.e.*, an anti-TSLC1 antibody) or nucleic acid probe specific for TSLC1 may be used to detect the presence of TSLC1 polypeptide (using antibody) or *TSLC1* polynucleotide (using nucleic acid probe) in biological fluids or tissues. Oligonucleotide primers based on any coding sequence region and regulatory sequence region in the *TSLC1* sequence are useful for amplifying DNA, for example by PCR.

[0048]   When using PCR primers, nucleic acid probes or restriction endonucleases, the 5' regulatory region and coding sequence of the *TSLC1* sequence is analysed.

[0049]   Any specimen containing a detectable amount of *TSLC1* polynucleotide or TSLC1 polypeptide antigen can be used. Nucleic acid can also be analyzed by RNA in situ methods that are known to those of skill in the art such as by *in situ* hybridization. Preferred samples for testing according to methods of the invention include such specimens as (cervical) smears and/or (cervical) biopsies and the like. Preferably, cytologically abnormal (cervical) smears and/or biopsies of high-grade (pre)malignant lesions are used as samples for testing. Although the subject can be any mammal, preferably the subject is human.

[0050]   The invention methods can utilize antibodies immunoreactive with TSLC1 polypeptide, the predicted amino acid sequence of which is available as GenBank Accession No. BAA75822, or immunoreactive fragments thereof. Antibody that consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations can be used. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler, et al., Nature, 256: 495,1975).

[0051]   The term antibody as used in this invention is meant to include intact molecules as well as fragments thereof, such as Fab and F (ab') 2, which are capable of binding an epitopic determinant on TSLC1.

[0052]   Monoclonal antibodies can be used in the invention diagnostic methods, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the monoclonal antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays that can utilize monoclonal antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the monoclonal antibodies of the invention can be done utilizing immunoassays that are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

[0053]   Monoclonal antibodies can be bound to many different carriers and used to detect the presence of TSLC1. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such using routine experimentation.

[0054]   In performing the assays it may be desirable to include certain "blockers" in the incubation medium (usually added with the labeled soluble antibody). The "blockers" are added to assure that non-specific proteins, proteases, or antiheterophilic immunoglobulins to anti-TSLC1 immunoglobulins present in the experimental sample do not cross-link or destroy the antibodies on the solid phase support, or the radiolabeled indicator antibody, to yield false positive or false negative results. The selection of "blockers" therefore may add substantially to the specificity of the assays described in the present invention. A number of nonrelevant (i. e., nonspecific) antibodies of the same class or subclass (isotype) as those used in the assays (e. g., IgGl, IgG2a, IgM, etc.) can be used as "blockers". The concentration of the "blockers" (normally 1-100 $\mu g/\mu L$) may be important, in order to maintain the proper sensitivity yet inhibit any unwanted interference by mutually occurring cross-reactive proteins in the specimen.

[0055]   In using a monoclonal antibody for the in vivo detection of antigen, the detectably labeled monoclonal antibody is given in a dose that is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to enable detection of the site having the TSLC1 antigen for which the monoclonal antibodies are specific. The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having TSLC1 is detectable compared

to the background, depending upon the in vivo imaging or detection method employed, such as MRI, CAT scan, and the like. Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

[0056]   As a rule, the dosage of detectably labeled monoclonal antibody for *in vivo* diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. The dosage of monoclonal antibody can vary from about 0.001 mg/m^2 to about 500 mg/m^2, preferably 0.1 mg/m^2 to about 200 mg/m^2, most preferably about 0.1 mg/m^2 to about 10 mg/m^2. Such dosages may vary, for example, depending on whether multiple injections are given, tumor burden, and other factors known to those of skill in the art.

[0057]   For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay that is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for in vivo diagnosis is that the half-life of the radioisotope be long enough so that it is still detectable at the time of maximum uptake by the target, but short enough so that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for in vivo imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may be readily detected by conventional gamma cameras.

[0058]   For *in vivo* diagnosis, radioisotopes can be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions that can be bound to the monoclonal antibodies of the invention are ^{111}In, ^{97}Ru, ^{67}Ga, ^{68}Ga, ^{72}As, ^{89}Zr, and ^{201}Tl.

[0059]   A monoclonal antibody useful in the invention methods can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements that are particularly useful in such techniques include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr and ^{56}Fe.

[0060]   Other, *e.g. ex vivo,* diagnostic methods for the detection of TSLC1 production, *TSLC1* gene expression or disorders therein, include methods wherein a sample for testing is provided, which sample comprises a cell preparation from cervical or other tissue. Preferably such samples are provided as smears. In order to provide for efficient testing schemes, cytologically abnormal (cervical) smears and/or biopsies of high-grade (pre)malignant lesions are used as samples for testing.

[0061]   A tissue sample obtained from a mammal, preferably a human, is suitably pretreated to allow contact between a target cellular component of a test cell comprised in said sample with a reagent that detects TSLC1 and detecting a reduction in the TSLC1 as compared to that of a comparable normal cell. Samples may be mounted on a suitable support to allow observation of individual cells. Examples of well-known support materials include glass, polystyrene, polypropylene, polyethylene, polycarbonate, polyurethane, optionally provided with layers to improve cell adhesion and immobilization of the sample, such as layers of poly-L-lysine or silane. Cervical smears or biopsies may for instance be prepared as for the Papanicolaou (Pap) test or any suitable modification thereof as known by the skilled person, and may be fixed by procedures that allow proper access of the reagent to the target component. If storage is required, routine procedures use buffered formalin for fixation followed by paraffin embedding, which provides for a well-preserved tissue infrastructure. In order to allow for immunohistochemical or immunofluorescent staining, the antigenicity of the sample material must be retrieved or unmasked. One method of retrieving the antigenicity of formaldehyde cross-linked proteins involves the treatment of the sample with proteolytic enzymes. This method results in a (partial) digest of the material and mere fragments of the original proteins can be accessed by antibodies.

[0062]   Another method for retrieving the immunoreactivity of formaldehyde cross-linked antigens involves the thermal processing using heat or high energy treatment of the samples. Such a method is described in e.g. U.S. Pat. No. 5,244,787. Yet another method for retrieving antigens from formaldehyde-fixed tissues is the use of a pressure cooker, either in combination with a microwave or in the form of an autoclave, such as described in e.g. Norton, 1994. J. Pathol. 173(4):371-9 and Taylor et al. 1996. Biotech Histochem 71(5):263-70.

[0063]   Several alternatives to formaldehyde may be used, such as ethanol, methanol, methacarn or glyoxal, citrated acetone, or fixatives may be used in combination. Alternatively, the sample may be air-dried before further processing.

[0064]   In order to allow for a detection with nucleic acid probes, the sample material must be retrieved or unmasked in case of formalin fixed and paraffin embedded material. One method involves the treatment with proteolytic enzymes and a postfixation with paraformaldehyde. Proteolytic digestion may be preceded by a denaturation step in HCl. This method results in a (partial) digest of the material allowing the entry of probes to the target. No specific unmasking procedures are required in case of non-formalin fixed material, e.g. frozen material. Prior to hybridisation samples can be acetylated by treatment with triethanolamine buffer.

[0065]   The nucleic acid probes or antibodies are then contacted with the sample material in a suitable buffer and permitted to specifically hybridize or bind to their nucleic acid or protein target. Upon specific binding of the nucleic

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acid probes or antibodies to the target components, labeled probes and/or antibodies may be detected by such methods as confocal laser scanning microscopy, bright field microscopy, flow cytometry optionally in combination with fluorescence associated cell sorting, or modifications of these techniques, which are well known to the person skilled in the art.

[0066] In one embodiment of a method of the invention an increased methylation of the *TSLC1* promoter in the test cell and/or reduced production of TSLC1 in the test cell is detected as compared to the comparable normal cell.

[0067] The present invention also provides methods for treating a subject with HPV-induced invasive cancers associated with modification of TSLC1 production, or indicative thereof, comprising administering to a subject with the cancer a therapeutically effective amount of a reagent that increases *TSLC1* expression. In HPV-induced invasive cancers associated with tumor suppressor lung cancer 1 (TSLC1), the *TSLC1* nucleotide sequence is underexpressed as compared to expression in a normal cell, therefore, it is possible to design appropriate therapeutic or diagnostic techniques directed to this sequence. Thus, nucleic acid sequences that increase *TSLC1* expression at the transcriptional or translational level can be used and, for example, nucleic acid sequences encoding TSLC1 (sense) could be administered to the subject with the HPV-induced invasive cancer, such as invasive cervical cancer.

[0068] The term "invasive cervical cancer" denotes malignant as well as premalignant cell populations, which often appear to differ from the surrounding tissue both morphologically and genotypically. These disorders are found to be associated with absence of or reduced expression of *TSLC1*. Essentially, any test cell abnormality that is etiologically linked to expression of *TSLC1* could be considered susceptible to treatment using methods of the present invention that employ a reagent to increase *TSLC1* expression.

[0069] Increased *TSLC1* expression may be attained by suppression of methylation of *TSLC1* polynucleotide when *TSLC1* is under-expressed. When, upon diagnosis according to the present invention, the HPV-induced cancer detected is associated with TSLC1 expression, such methylation suppressive reagents as 5-azacytadine can be introduced to a cell. Alternatively, when, upon diagnosis according to the present invention, the invasive cervical cancer detected is associated with under-expression of TSLC1 polypeptide, a sense polynucleotide sequence (the DNA coding strand) encoding TSLC1 polypeptide, or 5' regulatory nucleotide sequences (i. e., promoter) of *TSLC1* in operable linkage with *TSLC1* polynucleotide can be introduced into the cell. Demethylases known in the art could also be used to remove methylation.

[0070] The present invention also provides gene therapy for the treatment of HPV-induced cancer associated with modification of TSLC1 production. Such therapy would achieve its therapeutic effect by introduction of the appropriate *TSLC1* polynucleotide that contains a TSLC1 structural gene (sense), into cells of subjects having HPV-induced cancer. Schemes and procedure for effecting gene therapeutic treatment are known in the art, e.g. in WO 02/14557. Delivery of sense *TSLC1* polynucleotide constructs can be achieved by using an expression vector or by using a colloidal dispersion system, preferably a recombinant expression vector, said recombinant expression vector preferably being a plasmid, a viral particle or a phage.

[0071] The polynucleotide sequences used in the methods of the invention may be the native, unmethylated sequence or, alternatively, may be a sequence in which a nonmethylatable analog is substituted within the sequence. Preferably, the analog is a nonmethylatable analog of cytidine, such as 5-azacytadine. Other analogs will be known to those of skill in the art. Alternatively, such nonmethylatable analogs could be administered to a subject as drug therapy, alone or simultaneously with a sense structural gene for *TSLC1* or sense promoter for *TSLC1* operably linked to *TSLC1* structural gene. Preferably the *TSLC1* polynucleotide used in the invention methods is derived from a mammalian organism, and most preferably from human.

[0072] Allelic loss of the *TSLC1* locus (or LOH) can be detected by PCR amplification of polymorphic sequences flanking the *TSLC1* gene. Both DNA from test cells and from healthy cells of the same individual are PCR amplified. The two PCR fragments representing both alleles are separated on a polyacrylamide gel or by capillary electrophoresis. PCR products derived from DNA of a healthy cells and of test cells are compared , wherein loss of one of two PCR fragments in test cells is indicative of an allelic loss/genetic deletion of *TSLC1* locus.

[0073] The present invention also provides a kit of parts for use in a method of detecting HPV-induced invasive cancers and their precursor lesions associated with tumor suppressor lung cancer 1 (TSLC1) in test cells of a subject. Such a kit may suitably comprise a brush or spatula to take a (cervical) scrape together with a container filled with collection medium to collect test cells. Alternatively, a sampling device consisting of an irrigation syringe, a disposable female urine catheter and a container with irrigation fluid will be included to collect cervical cells by cervico-vaginal lavage.

[0074] A kit according to the present invention may comprise primers and probes for the detection of TSLC1 promoter methylation, for the detection of allele losses at chromosome 11q23.2 or for the detection of TSLC1 mRNA expression. In another embodiment, a kit according to the invention may comprise antibodies and reagents for the detection of TSLC1 protein expression in cervical scrapes or tissue specimens.

[0075] A kit of parts according to the invention comprises means for the detection of *TSLC1* promoter methylation or *TSLC1* expression, such as TSLC1-specific antibodies, methylation-sensitive restriction enzymes, or probes or primers capable of hybridising to the nucleotide sequence of Fig. 1.

[0076] In yet another alternative embodiment of a kit of the invention the means for the detection of *TSLC1* promoter methylation or *TSLC1* expression may be combined with means for the detection of HPV infection, preferably for the detection of HPV infection of the high-risk type. Such means may comprise HPV-specific primers or probes as are known in the art.

[0077] The present invention will now be illustrated by way of the following, non limiting examples.

EXAMPLES

Example 1a. Frequent *TSLC1* silencing in cervical carcinoma cell lines

[0078] *TSLC1* mRNA expression levels were measured in 11 cervical carcinoma cell lines by real time quantitative RT-PCR using Lightcycler technology (Roche). Compared to normal primary epithelial cells, *TSLC1* mRNA was undetectable in 8 of the 11 cell lines and severely reduced in another 2 cell lines, showing that TSLC1 downregulation is apparent in 91% (10/11) of cervical carcinoma cell lines analysed.

[0079] On the other hand, *TSLC1* expression was still abundant in HPV-immortalized cells. These HPV-immortalized cells are not yet tumorigenic and have previously been shown to be representative of premalignant cervical lesions in vivo.

[0080] Subsequently, the analysis of the mechanisms underlying *TSLC1* downregulation was undertaken. Besides genetic events, i.e. deletions and inactivating mutations, epigenetic events may result in gene silencing, as has been described for lung cancers [Kuramochi et al., 2001].

Example 1b. Role of methylation in *TSLC1* silencing in cervical cancer cells

[0081] To assess whether a methylating event was underlying *TSLC1* silencing in cervical cancer cells, the cell lines SiHa, HeLa and CaSki were treated with the methylation inhibitor 5-aza-cytidine. TSLC1 mRNA levels were compared to expression levels in primary epithelial cells, which was set to a 100%. Upon 5 to 7 days of incubation with 5-azacytidine *TSLC1* expression levels were upregulated from 0% to 26% in SiHa, and from 4% to 70% and 0% to 33% in HeLa and CaSki cells, respectively. These data indicate that in all these 3 cell lines TSLC1 downregulation results, at least in part, from a methylating event.

[0082] Next, *TSLC1* promoter methylation by radioactive bisulfite sequencing was studied. It was found that all 6 CpG sites sequenced were methylated in 9/11 cervical cancer cell lines. Except for one cell line, no wild type sequences were detected in these cell lines, indicating that *TSLC1* promoter methylation was clonal. In the remaining 2 cell lines none of the CpG sites were methylated. Except for one cell line, *TSLC1* promoter methylation was correlated to reduced or undetectable *TSLC1* mRNA expression. No *TSLC1* promoter methylation was detected in 4 isolates of normal epithelial cells and in the non-tumorigenic HPV-immortalized cells.

Example 1c. Role of a chromosomal deletion in *TSLC1* silencing

[0083] To analyse whether a chromosomal deletion attributed to *TSLC1* silencing loss of heterozygosity (LOH) analysis was performed using 8 polymorphic markers flanking the TSLC1 gene. Normal DNA derived from either lymphoblasts or fibroblasts of 8 cervical carcinoma cell lines was available. In 3 (38%) of these cell lines an allelic loss at 11q23.2 was apparent. All 3 cell lines revealed *TSLC1* promoter hypermethylation and absence of detectable *TSLC1* mRNA, suggesting that allelic deletion combined with promoter hypermethylation underlied complete gene silencing.

[0084] Analysis on the HPV immortalized cell lines showed an LOH at 11q23.2 in all immortal passages of one of the four cell lines. However, *TSLC1* expression was still detectable in these passages and no promoter methylation was found, indicating that the retained allele is still actively transcribed. No allelic loss at 11q23 was detected in the other three cell lines.

[0085] Taken together, these data show that *TSLC1* silencing in cervical cancer cells may result from 1) promoter methylation of one allele combined with deletion of the other allele as found in 3/8 cell lines, 2) promoter methylation without an allelic loss, suggesting that either both alleles are hypermethylated or that one is mutated, as was found in two cell lines, or 3) other yet unknown mechanisms, as was found in two cell lines.

Example 2. TSLC1 suppresses anchorage independent growth in vitro and tumorigenicity in nude mice

[0086] Malignant transformation of hr-HPV infected epithelial cells in vitro has been shown to proceed via the subsequent acquisition of 1) an immortal phenotype and 2) an anchorage independent phenotype, which can be measured by the growth of cells in soft agarose.

[0087] To test whether TSLC1 silencing is functionally involved in the development of cervical cancer, the TSLC1

coding sequence was transfected in the cervical cancer cell line SiHa in which endogenous TSLC1 expression was undetectable. Re-expression of TSLC1 in these TSLC1 mRNA negative SiHa cells resulted in a highly reduced growth in soft agarose, which is a measure for anchorage independence, whereas the growth in normal monolayer was un-affected. Moreover, re-expression of TSLC1 in SiHa cells resulted in a suppression of tumor growth in nude mice in two of four SiHa/TSLC1 transfectants and tumors that did arise from the SiHa/TSLC1 transfectants grew slower than the controls.

[0088] In conclusion, these results show that TSLC1 can suppress both anchorage independent and tumorigenic growth of cervical cancer cells, leaving immortality and proliferation unaffected.

Example 3. Silencing of TSLC1 in cervical tissue specimens

[0089] To determine whether and at what stage during cervical carcinogenesis TSLC1 silencing occurs in vivo, the methylation status of the TSLC1 promoter was analyzed in cervical tissue specimens.

[0090] Since the tissue specimens consist of an admixture of normal stromal components and abnormal cells the sensitivity of the assay to detect methylated CpG islands in a background of normal unmethylated DNA was determined. By analysing a dilution series of SiHa DNA (methylated TSLC1 promoter) in DNA derived from primary keratinocytes (unmethylated TSLC1 promoter), it was found that as low as 5% of methylated DNA in a background of unmethylated DNA could still be detected with high reliability, using radioactive bisulfite sequencing on a Genomyx device.

[0091] Using this method none of 15 normal cervical epithelial biopsies revealed TSLC1 promoter methylation. Sim-ilarly, TSLC1 promoter methylation was undetectable in all low grade CIN lesions (n=10). Of 30 high grade CIN lesions 35% showed methylation of the TSLC1 promoter. In addition, a total of 50 cervical squamous cell carcinoma sections were analysed, 58% (29/50) of which showed TSLC1 promoter methylation. Thus, TSLC1 promoter methylation ap-pears to be a rather frequent event in cervical squamous cell carcinomas and occurs late during the multistep sequence of carcinogenesis. Since in addition to TSLC1 promoter hypermethylation other alterations, including an allelic loss at 11q23.2, may contribute to silencing of TSLC1 the actual percentage of TSLC1 silencing in cervical carcinomas is likely to be even higher than 58%.

Example 4. Detection of TSLC1 promoter methylation in cervical smears

[0092] In a small pilot study it was assessed whether the detection of TSLC1 alterations can be applied to cervical smears and as such be used as a marker in cervical cancer screening programs.

[0093] For this, TSLC1 promoter methylation was analysed in archival smears of women who developed cervical cancer. These scrapes were derived from a retrospective case-control which was designed to determine the value of hr-HPV testing to signal false negative cervical smears in women who developed cervical cancer and to assess whether hr-HPV is present in normal smears preceding cervical cancer (Zielinski et al., 2001; Br J Cancer 85, 398-404). Analysis of 9 index smears, i.e. smears taken at the time of cervical cancer diagnosis and classified as Pap4 or Pap5 (i.e. suspect for carcinoma in situ/invasive cancer), as well as the 9 associated cervical carcinoma biopsies, showed that TSLC1 promoter methylation could be detected in smears of women with a TSLC1 promoter methylation positive cancer biopsy. No TSLC1 promoter methylation was detected in control smears (n=12).

[0094] As a follow up it was determined whether the detection of TSLC1 promoter methylation cannot only provide a diagnostic marker to detect cervical cancer but may also provide a marker for risk assessment of premalignant cervical lesions. Hereto, the same patient group was studied as described above and archival cervical smears were analysed which were taken 1-2 years before cervical cancer was diagnosed and which were classified as Pap3a2/3b, i.e. moderate/severe dyskaryosis. In one patient, TSLC1 promoter methylation could already be detected in a cancer preceding smear, which was taken 1 year prior to cervical cancer diagnosis.

SEQUENCE LISTING

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<120> Detection of HPV-induced invasive cancers and their
precursor lesions with invasive potential

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Claims

1. A method of detecting an HPV-induced invasive cancer or precursor lesion thereof associated with tumor suppressor lung cancer 1 (TSLC1) in a subject in need thereof, said method comprising contacting a cell component of a test cell of the subject with a reagent that detects the level of the cell component in the test cell and determining a modification in the level of the cell component in the test cell as compared with a comparable healthy cell, wherein the cell component indicates the level of TSLC1 in the cell and the modification indicates the presence of an HPV-induced invasive cancer or precursor lesion thereof.

2. Method according to claim 1, wherein said HPV-induced invasive cancer or precursor lesion thereof is invasive cervical cancer or a premalignant cervical lesion with invasive potential.

3. Method according to claim 1 or 2, wherein said HPV-induced invasive cancer is a high-risk HPV-induced invasive cancer.

4. Method according to any one of the preceding claims, wherein the modification is a decrease in the level of TSLC1.

5. Method according to any one of the preceding claims, wherein the cell component is a nucleic acid associated with production of TSLC1 polypeptide and the reagent targets the nucleic acid in the test cell, said nucleic acid preferably encoding the TSLC1 and regulatory regions.

6. Method according to claims 5, wherein the nucleic acid is RNA, preferably mRNA.

7. Method according to any one of the preceding claims, wherein the reagent is a restriction endonuclease, preferably a methylation sensitive restriction endonuclease.

8. Method according to claim 5 or 6, wherein the reagent is a nucleic acid probe or primer that binds to the nucleic acid, said nucleic acid probe or primer preferably having a detectable label.

9. Method according to claim 8, wherein the nucleic acid probe has a nucleotide sequence selected from the group consisting of:

    a) a polynucleotide sequence capable of hybridizing under stringent conditions to the 5' regulatory region or the coding region of the TSLC1 sequence as set forth in Figure 1;
    b) a polynucleotide having at least 70% identity to the polynucleotide of a);
    c) a polynucleotide complementary to the polynucleotide of a); and
    d) a polynucleotide comprising at least 15 bases of a nucleotide of a) or b).

10. Method according to any one of the claims 1-4, wherein the cell component is a polypeptide and the reagent targets the polypeptide in the test cell, said polypeptide preferably being TSLC1 and said reagent preferably being an anti-TSLC1 antibody.

11. Method according to any one of the claims 1-5, wherein said detecting evaluates the methylation status of the TSLC1 promoter.

12. A method of detecting HPV-induced invasive cancer or precursor lesion thereof associated with tumor suppressor lung cancer 1 (TSLC1) in a subject in need thereof, said method comprising contacting a target cellular component of a test cell with a reagent that detects TSLC1 and detecting a reduction in the TSLC1 as compared to that of a comparable normal cell, preferably in said detection an increased methylation of the TSLC1 promoter in the test cell and/or a reduced production of TSLC1 in the test cell as compared to the comparable normal cell is determined.

13. Method according to claim 12, wherein the target cellular component is nucleic acid.

14. Method according to claim 13, wherein the nucleic acid is RNA, preferably mRNA.

15. Method according to claim 12, wherein the target cellular component is a protein.

16. Method according to claim 13 or 14, wherein the reagent is a nucleic acid probe or primer that binds to TSLC1.

17. Method according to claim 15, wherein the reagent is an anti-TSLC1 antibody.

18. Method according to any one of the claims 12-17, wherein the subject has loss of heterozygosity at chromosome 11q23.

19. A method of treating HPV-induced invasive cancers and their precursor lesions associated with modification of TSLC1 production in cells in a subject in need thereof, said method comprising contacting said cells of a patient suffering from said cancer with a therapeutically effective amount of a reagent that increases TSLC1 level in said cells.

20. Method according to claim 19, wherein the reagent is a polynucleotide sequence comprising a TSLC1 sense polynucleotide sequence, preferably said polynucleotide is the native, unmethylated TSLC1 sense sequence.

21. Method according to claim 20, wherein a nonmethylatable analog is substituted for cytidine within the TSLC1 sense sequence, said nonmethylatable analog preferably being is 5-azacytadine.

22. Method according to claim 20 or 21, wherein said polynucleotide sequence is contained in an expression vector, said expression vector preferably being a plasmid, a viral particle or a phage.

23. Use of a molecular diagnostic marker for the detection of HPV-induced invasive cancers and their precursor lesions associated with tumor suppressor lung cancer 1 (TSLC1), wherein said marker indicates *TSLC1* promoter methylation, expression of mRNA associated with production of TSLC1 polypeptide and/or allelic loss of chromosome 11q23.

24. Kit of parts for use in a method of detecting HPV-induced invasive cancers and their precursor lesions associated with tumor suppressor lung cancer 1 (TSLC1) in test cells of a subject, said kit comprising means to collect cervical cells and means for the detection of *TSLC1* promoter methylation or *TSLC1* expression.

25. Kit of parts for use in a method of detecting HPV-induced invasive cancers and their precursor lesions associated with tumor suppressor lung cancer 1 (TSLC1) in test cells of a subject, said kit comprising means for the detection of *TSLC1* promoter methylation or *TSLC1* expression and means for the detection of HPV infection.

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Figure 1

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number

EP 03 07 5928

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
D,X	WO 02 14557 A (YOSHINORI MURAKAMI ; REEVES ROGER HARPER (US); UNIV JOHNS HOPKINS M) 21 February 2002 (2002-02-21) * page 15, line 12 - line 25 *	24	C12Q1/68 A61K38/17
A	*the whole document, in particular claim 4*	1-23	

X	PULIDO HUGO ARIAS ET AL: "Identification of a 6-cM minimal deletion at 11q23.1-23.2 and exclusion of PPP2R1B gene as a deletion target in cervical cancer" CANCER RESEARCH, vol. 60, no. 23, 1 December 2000 (2000-12-01), pages 6677-6682, XP002261292 ISSN: 0008-5472 * page 6677, column 2, paragraphs 1,2 *	23	

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TECHNICAL FIELDS SEARCHED (Int.Cl.7)

C12Q
A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
MUNICH	18 November 2003	Schmitt, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

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Office

**INCOMPLETE SEARCH
SHEET C**

Application Number

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Although claims 1-18 encompass diagnostic methods practised on the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Although claims 19-22 are directed to methods of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Claim(s) searched completely:
        23-25

Claim(s) searched incompletely:
        1-22

Reason for the limitation of the search (non-patentable invention(s)):

Article 52 (4) EPC - Method for treatment of the human or animal body by therapy
Article 52 (4) EPC - Method for treatment of the human or animal body by surgery

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Further limitation of the search

Claim(s) searched completely:
        12-18, 20-22

Claim(s) searched incompletely:
        1-11, 19, 23-25

Reason for the limitation of the search:

1) Claim 1 relates to a method of detecting an HPV-induced invasive cancer comprising contacting a cell component with a reagent, wherein the cell component is only defined in term of its function (i.e. the cell component indicates the level of TSLC1 in the cell). These functional features are however not sufficient to characterize said cell component so as to allow the skilled person to clearly and unambiguously understand the scope of said claim (Article 84 EPC). This lack of clarity is such as to render a meaningful search over the whole claimed scope impossible. Consequently, the search has been carried out for those parts of the claim which appear to be clear in view of the description, namely those parts relating to the cell component being the TSLC1 gene, TSLC1 gene transcripts or proteins encoded by said transcripts (page 10, lines 1-21).
The search of dependent claims 2-11 has been carried out accordingly.

2) Furthermore, claim 19 relates to a method of treatment based on a reagent that is only defined in term of its function (i.e. the reagent increases TSLC1 level). This functional feature is however not sufficient

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to characterized said reagent so as to allow the skilled person to
clearly and unambiguously understand the scope of said claim (Article 84
EPC). This lack of clarity is such as to render a meaningful search over
the whole claimed scope impossible. Consequently, the search has been
limited to the reagent being a nucleic acid encoding TSLC1 or a reagent
that suppresses methylation of TSLC1 polynucleotide (pages 19-20).

3) Claim 23 relates to the use of a molecular diagnostic marker for the
detection of HPV-induced invasive cancers, wherein said diagnostic marker
is only defined in term of its function (i.e. the diagnostic marker
indicates TSLC1 promoter methylation, expression of mRNA associated with
production of TSLC1 polypeptide and/or allelic loss of chromosome 11q23).
This functional feature is however not sufficient to characterize said
marker so as to allow the skilled person to clearly and unambiguously
understand the scope of said claim (Article 84 EPC). This lack of clarity
is such as to render a meaningful search over the whole claimed scope
impossible. Consequently, the search has been carried out for those parts
of the claim which, in view of the description (page 8, line 18-page 9,
line 11 and page 10, lines 1-11), appear to be clear, namely those parts
relating to the use of TSLC1 promoter methylation, expression of TSLC1
mRNA or polypeptide and/or allelic loss of chromosome 11q23 as a
molecular diagnostic marker for detection of HPV-induced invasive cancers
and their precursor lesions.

4) Claims 24 and 25 relate to kit of parts for use in a method of
detecting HPV-induced invasive cancers and their precursor lesions
comprising means only defined in term of their function (i.e. means for
the detection of TSLC1 promoter methylation or TSLC1 expression or means
for the detection of HPV infection). These functional features are
however not sufficient to characterize clearly said means (Article 84
EPC). A meaningful search over the whole claimed scope is therefore
impossible and the search has been carried out for those part of the
claims which appear clear in view of the description (page 21, lines
1-15), namely those parts relating to means for the detection of TSLC1
promoter methylation or TSLC1 expression being primers or probes capable
of hybridizing to the TSLC1 nucleotide sequence of Fig.1, TSLC1-specific
antibodies or methylation-sensitive restriction enzymes and means for the
detection of HPV infection being HPV-specific primers or probes.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number

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	DOCUMENTS CONSIDERED TO BE RELEVANT		CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	LIST HEINZ-JOACHIM ET AL: "Methylation sensitivity of the enhancer from the human papillomavirus type 16" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 16, 1994, pages 11902-11911, XP002261476 ISSN: 0021-9258 * abstract * * page 11902, column 1 - column 2, paragraph 3 * * page 11903, column 2, paragraph 2 * * figure 6 *	25	
A	WO 99 29890 A (DIGENE CORP ;LORINCZ ATTILA T (US)) 17 June 1999 (1999-06-17) * page 17, line 17 - line 22 * * page 18, line 20 - page 19, line 4 * * claims 6,7 * * the whole document *	1-23	**TECHNICAL FIELDS SEARCHED** (Int.Cl.7)
A	FUKUHARA HIROSHI ET AL: "Promoter methylation of TSLC1 and tumor suppression by its gene product in human prostate cancer" JAPANESE JOURNAL OF CANCER RESEARCH, vol. 93, no. 6, June 2002 (2002-06), pages 605-609, XP002260474 ISSN: 0910-5050 * the whole document *	1-25	

EPO FORM 1503 03.82 (P04C10)

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**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**   EP 03 07 5928

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
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18-11-2003

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			CA	2419529 A1	21-02-2002
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			WO	0214557 A1	21-02-2002
			US	2003165974 A1	04-09-2003
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EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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